(NASA-CR-138075) INVESTIGATIONS IN SPACE-RELATED MOLECULAR BIOLOGY Final Report (Chicago Univ.) 22 p HC
CSCL 06C
N74-20715
Unclas
G3/04 16966

3-27-74

FINAL TERMINATION REPORT
NASA NGL 14-001-012

INVESTIGATIONS IN SPACE-RELATED MOLECULAR BIOLOGY

H. Fernandez-Moran, M.D., Ph.D.
A.N. Pritzker Professor of Biophysics
Division of Biological Sciences and Pritzker School of Medicine
The University of Chicago, Chicago, Illinois 60637

GENERAL SUMMARY

With funds provided by NASA NGL 14-001-012 during the past eighteen months, correlated high voltage, low temperature electron microscopy and diffraction studies of both terrestrial and extraterrestrial specimens have been carried out by a professional research team in our specially-designed laboratories at The University of Chicago.

We have also continued collateral developmental work to provide increasing amounts of information from hydrated and dried biological specimens examined under conditions of increased specimen stability and significantly reduced radiation damage, thermal noise and contamination. This research has centered on:

a. Continued development and testing of new low temperature instrumentation and preparation techniques, such as diamond knife cryo-ultramicrotomy and operation of the world's first large-scale Collins closed-cycle superfluid helium refrigeration system. This unit is fully integrated with the 200 kV microscope. Specimen cooling at 1.8° to 4.2°K consistently permits resolutions of 3Å to 10Å in both bright and dark field. Specially-adapted vacuum-tight microchambers designed for use at liquid helium temperatures have been used in low temperature studies of ice-crystal formation, hydrated membranes, virus structures, bacteriophages, lunar samples and new types of organometallic superconducting compounds with consistent resolutions on the order of 8Å to 16Å.

b. Development of new highly sensitive polymer-based films to record high resolution color electron micrographs for the first time. Results of comparative analyses show heretofore unobserved structural details in both organic and inorganic specimens and provide a quantitative evaluation of electron beam interaction with the specimen under certain conditions.

c. Development and refinement of new types of high resolution plastic lenses and reflecting objectives of the aspherical type which can be used in portable information storage and retrieval systems and perhaps as a source of harnessing the sun's energy.

SPECIFIC AIMS

Based on earlier work in the field of instrumentation development and ultrastructural research funded in part by the National Aeronautics and Space Administration, during the past eighteen months we have been able to attain many of the goals set forth in our last renewal application for NASA NGL 14-001-012. Among these have been:

A. Further refinement of improved instrumentation and preparation techniques for high resolution, high voltage cryo-electron microscopy.

1. Diamond Knife Cryo-Ultramicrotomy. In the course of this project, we have been able to produce precise thin sections of various lunar samples and biological specimens at liquid helium temperatures using a special cryo-ultramicrotome and associated diamond knife (1). In this manner, it is possible to preserve the inherent hydrated components of biological specimens for study by electron microscopy and diffraction techniques.

Most of our direct knowledge of lamellar systems and cell fine structure to date is based on electron microscopy of osmium-fixed specimens which are deprived of their hydrated components during preparation and examination. By combining diamond knife cryo-ultramicrotomy methods with examination at low temperatures in specially-designed vacuum-tight microchambers (2), we are now able to detect the non-lipid myelin components, such as water, proteins, polysaccharides, salts and nucleic acids, under conditions approaching the native state. This work may well provide evidence for the modern picture of membranes as dynamic structures--mosaics of patches of lipid, areas of protein, and carbohydrate sites where the sugar end of glycoprotein sits on the outer membranes (3).

Based on this experience, and in view of the unique properties of the diamond knife and ultramicrotome (1), it is now feasible to think in terms of constructing a cryo-ultramicrotome and diamond knife small enough to fit directly into the microscope stage. This instrument could then be manipulated from outside so that preparation and examination of specimens could be carried out in one single system and in one coordinated operation.

2. High Resolution Electron Microscopy. During the past decade, our skilled inter-disciplinary research team has helped to construct and develop the only functioning center for high resolution electron microscopy in the world capable of operating practically on a 24-hour basis free from ambient perturbations. In the course of establishing this singular national facility, we have developed, tested and routinely applied several modified high resolution electron microscopes (75 kV to 200 kV), including a 200 kV instrument integrated with the Collins closed-cycle superfluid helium refrigerator which can be operated at 1.8° to $4.2^{\circ}K$ with superconducting lenses (4).

Several of these microscopes are fitted with specially-designed improved point cathode sources for stable, coherent microbeam illumination of high brightness, small spot size and low energy spread.

In addition, they incorporate short focal length objective lenses (1.5 mm) to maximize instrument performance and to make enhanced dark field imaging practical (5).

High field magnetic superconducting lenses (27,220 ampere-turns) have been developed in our laboratory and used routinely to increase instrument resolution (6,7). Operating in the persistent current mode, consistent resolutions of 10Å to 20Å were achieved in an early series of experiments during longer exposure times at lower beam intensities, thus reducing specimen radiation damage.

By combining the advantages of these new instrumental developments, we can now achieve resolutions on the order of 3Å to 10Å consistently with significantly reduced specimen damage, contamination, thermal noise and electron optical aberrations at temperatures in the liquid helium range (i.e. 1.8^{o} to 4.2^{o}K). This work was the subject of an extensive series of papers and lectures which I presented during various international colloquia in Germany in 1972. (8,9).

We have also build, but not yet tested, a superconducting objective lens of 100,000 ampere-turns designed for work in the superfluid helium cryostat of the high voltage (200 kV) microscope (4). This promises to provide us with an integrated superconducting cryo-electron microscope system which would permit unmatched stability of lens excitation current and high voltage (5 kV to 10 MeV), correction of spherical lens aberrations, including trapped flux, phase zone apertures, and coherent electron beam point cathodes operating in cryogenic ultra-high vacuums to decisively reduce radiation damage, contamination and thermal noise while enhancing image quality with an optimized image intensifier operating at liquid helium temperatures (10,11).

The importance of this research has been independently confirmed by scientists from several countries. Among the papers presented at the Fifth European Conference on Electron Microscopy in 1972, the following are of special significance in regard to our current work in this area: "The Effect of Cooling on the Diffraction Pattern of Beam-Sensitive Specimens" by M. G. Dodd (12); "Superconducting Lenses" by A. Septier (13); and "Reduced Radiation Damage of Halogenated Copper-Phthalocyanine" by Uyeda, Kobayashi, Ohara, Watanabe, Taoka and Harada (14).

3. Improved Dark Field Imaging Techniques. During the past eighteen months, we have been able to extend earlier work by combining the improved dark field capacity of the 75 kV microscope and new dark field imaging methods developed in our laboratory (15,16). This has permitted us to attain resolutions of 4Å to 10Å repeatedly in unstained biological and extraterrestrial specimens from DNA to lunar pyroxenes and organometallic superconducting compounds at temperatures ranging from 290^{o}K to 1.8^{o} and 4.2^{o}K using short focal length objective lenses and specially designed thin-film condenser apertures (17).

These high resolution dark field studies have demonstrated that unstained or lightly stained specimens when shadow cast with thin (ca. 50Å) grainless carbon films reveal new details which cannot be seen in the corresponding shadow cast bright field images. This is important because it demonstrates the possibility of achieving the highest instrumental resolutions (i.e. 3Å to 7Å) while at the same time partially protecting the specimens against denaturing radiation damage (16). Even allowing for unavoidable radiation damage, such as chemical deterioration, and its accompanying morphological changes, native ultrastructure or its meaningful equivalent picture can be recorded in the form of grainless replications obtained by shadow casting with carbon films prior to electron microscopic observation (18).

4. High Voltage (200 kV) Electron Microscopy. Based on the pioneering work of Drs. G. Dupouy and F. Perrier in France, there are several advantages to be derived from high voltage electron microscopy from the biological and biomedical researchers' viewpoint. Among these are: 1) increased penetration power which permits examination of thicker preparations and specimen whole mounts, 2) improved dark field ability due to the decreased chromatic aberration, and 3) improved bright field resolution resulting from reduction of spherical aberration (19).

Working with Dr. Ronald W. Moses, Jr., one of the most outstanding electron optical theorists in the United States, who has been attached to our laboratory during eight months of this project, we have also been able to gain new insights into microscope design and operation at high voltages which may well open whole new areas of the instrument's application in both biological and extraterrestrial research. A summary of Dr. Moses' work in connection with our research group can be found in the paper "Procedures for Correcting Aberrations in the High Voltage Electron Microscope," which is soon to be published in PROCEEDINGS OF THE ROYAL SOCIETY, LONDON (20).

5. Collins Closed-Cycle Superfluid Helium Refrigerator. The work of Prof. Samuel C. Collins, who conceived and built a helium cryostat simpler in deisgn and more efficient than any previous liquifier, resulted in a reliable piece of standard equipment now in use throughout the world. Collins and his colleagues for the first time made large quantities of superfluid helium available by reproducibly achieving temperatures of 1.8° to 4.2°K with relative efficiencies close to 8% to 10% of the Carnot cycle. This is comparable with the efficiency ratings of machines operating at much higher temperatures (21).

The unique non-viscous flow properties of the superfluid helium and its singularly high thermal conductivity at such low temperatures demands that the entire system, including over 36 feet of transfer lines and a novel heat exchanger installed in parts of a 5-story building, be super-leak-proof. With the assistance of Prof. Collins, M. Streeter and R. Osburn of Cryogenic Technology, Inc., the large-scale Collins closed-cycle superfluid helium refrigeration unit has been fully integrated with the modified high voltage (200 kV) electron microscope in our laboratory (10).

This cryo-electron microscope system, which is the world's only functioning prototype, has been used routinely in over 170 successful experiments to significantly reduce specimen radiation damage, contamination and thermal noise during prolonged vibration-free examination of specimens at 1.8° to $4.2^{\circ}K$. At the same time, it makes it possible to combine these advantages with high penetration power, ultra-high vacuums, decreased spherical and chromatic lens aberrations and enhanced image contrast (4). At $1.9^{\circ}K$, this closed-cycle superfluid helium unit, with a 20-watt refrigeration capacity, can supply superfluid helium to the specially-designed microscope cryostat at rates of 5 to 8 liters per hour. With pre-cooling, the rate of liquification approaches 216 liters per day.

Preliminary data indicate the highest recorded resolutions at liquid helium temperatures have been attained in the course of this developmental work, carried out under NASA NGL 14-001-012 and USPHS GM 13243-08. At 1.8° to $4.2^{\circ}K$, consistent resolutions of 8Å to 10Å have been achieved under ideal conditions (3). This compares with similar resolutions of about 50Å at low temperatures a little over two years ago (22). In addition, this low temperature approach may make controlled manipulation of molecular structure under conditions of reduced entropy a real possibility--thus perhaps providing a rational theraputic approach which may be valuable in treating cancer cases and various genetic diseases.

In addition, we have observed prolonged maintenance of the cold temperatures at $4.2^{\circ}K$ in the cold stage of the microscope connected with the superfluid helium closed-cycle system for periods of up to 30 minutes after all the refrigeration equipment has been completely shut off. This unexpected result may have important implications for superconducting cables designed to transmit large blocks of electrical energy over long distances without loss (23).

This work was the subject of an extensive paper which I presented last October at the annual meeting of the Cryogenic Society of America and the American High Vacuum Society (4). Low temperature physicists and cryo-biologists attending repeatedly referred to our research as the only work of its kind now systematically underway in this country.

6. Enhanced Recording Media.

a. High Resolution Color Electron Micrographs and Synthetic Aperture Color Holograms. By either one of two different methods, using light- and electron-sensitive films respectively, high resolution color electron micrographs of various biological specimens can now be recorded for the first time. These films, based on earlier developments by several researchers (24,25,26), have been developed and tested in our laboratory. They present interference colors as a function of the image-dependent thickness variations in the emulsions.

To date, the best results have been obtained using a method developed by my associate Charles Hough and based on work by Lippmann in the mid-nineteenth century. Clean glass plates are coated with 1000Å thicknesses of aluminum. The aluminum surface is coated with a special dichromated emulsion and put in contact with the positive electron image plate, resulting in something like a photographic contact print. The aluminum plate is then exposed to high intensity mercury lamps, developed and dried. This color translation process converts normally indistinguishable shades of gray in high resolution black and white electron micrographs into clearly distinct color differences, thus revealing heretofore unobservable structural detail (3,27).

In the other method, the electron micrograph is recorded directly on new, essentially grainless, electron-sensitive films of silicone-based organic polymers, similar to those used by Kanaya in Japan (28). The recorded images can be observed as color images and the differences in mass-thickness of biological specimens estimated directly from the measured variations in color and photographic density. Using a precisely controlled microbeam to irradiate the specimen through a full series of color recordings, we can obtain progressive visual records of specimen radiation damage, an approach which may prove valuable for those working in the field of radiation research.

Perliminary tests, rated on performance scales from the National Bureau of Standards, indicate that these silver-free films have a resolving power in excess of 2000 lines per millimeter. This is a rating unmatched by any commercial film now being used. They also provide exceptionally high diffraction efficiency (i.e. 90%) combining both thickness modulation and spatial variations.

High resolution synthetic aperture color holograms can also be reconstructed from electron images recorded under special conditions. This may provide new insights into the three-dimensional relationships within the macromolecular complexes of the specimens, including viruses and virus particles.

b. Computer Enhancement. Computer enhancement of periodic images of stained and unstained catalase crystals has revealed new structural details of the subunits which can be correlated with low-angle electron diffraction studies. Using high resolution electron micrographs recorded on special thin films in our laboratory, R. Nathan has demonstrated that an extreme improvement in signal to noise ratio can be obtained by translating the image by the amount of periodicity and superimposing it upon itself. Images are then displayed where intensities are contoured (29).

7. Fly's Eye Lenses and Mirrors. Based on techniques developed in collaboration with Donald Powers of the Physical Sciences Developmental Workshop of The University of Chicago and Charles Hough, we have succeeded in developing and testing new types of miniature plastic multiple lenses and mirrors. These optical arrays, containing from 10 to 5,000 individual units of high quality per square inch, are

made of ultra-violet transmitting plastic using a high pressure molding process and precision molds generated by a computer controlled system.

Since the master mold required for producing lenses and mirrors of extremely high quality necessitates an ultra-smooth finish, the diamond knife can be used for attaining the high degree of precision demanded. (In a series of ultra-precise applications, the diamond knife has been used to cut optical quality finishes of up to 0.2 micro-inches without any additional polishing. Other researchers have also suggested the possibility of adapting the diamond knife to fully automated tooling machines, thus permitting the mass production of uniform, ultra-high finishes by remote control and markedly decreasing the cost of obtaining precision.)

Once the mold is made, the lenses and mirrors, of varying sizes and shapes, can be manufactured in one fully-automated embossing process with a saving equivalent to several orders of magnitude in material, manufacturing cost and testing.

Preliminary tests of these miniaturized lenses indicate they have exceptional optical characteristics, including very high resolving power on the order of 400 to 1000 lines per millimeter. They are also corrected for spherical and chromatic aberration, astigmatism and field distortion. The corresponding reflecting elements are also color corrected for all wavelengths of the visible, ultraviolet and infra-red spectrum.

These lenses and mirrors, together with the new types of organometallic recording media, have proved important in the design and construction of highly improved information storage and readout systems as will be discussed subsequently.

B. Going beyond this mere developmental phase of our work, we have, during the past eighteen months, <u>systematically applied these instruments and techniques to a wide variety of ultrastructural studies and research problems</u>, including:

1. <u>Continued participation in the electron microscopic and electron optical analysis and identification of lunar and other extraterrestrial material.</u>

Based on earlier work with Dr. Stefan S. Hafner and his colleagues of the Department of the Geophysical Sciences at The University of Chicago, my associates and I have worked closely with co-investigator Dr. David Virgo of the Carnegie Institution Geophysical Laboratories to carry out correlated investigations of lunar pyroxenes from Apollo 11, 12 14 and 15 specimens and reference samples from selected terrestrial sources.

a. <u>Apollo 11 Studies.</u> Pyroxenes from lunar rock 10044,25 (composition $En_{36}Fs_{34}Wo_{30}$) were cleaved and sectioned by diamond knife ultramicrotomy and mounted directly on ultra-thin carbon films or platinum or copper grids.

Standard (75 kV) and high voltage (200 kV) electron microscopy and diffraction studies of iron-rich separates from this rock sample revealed exceptionally regular, periodically-spaced dense bands ~100Å to 600Å wide (average width ca. 300Å). At the first Lunar Science Conference, we reported that these straight-edged bands, corresponding to single crystal domains, closely resembled images of magnetic domain walls seen in layers of ferromagnetic materials (30). Lattice spacings of 2.5Å could be detected within the bands, probably corresponding to d(200).

These findings are consistent with the observation of extensive exsolution lamellae as described by Radcliff, Bailey and Ross (31, 32,33). Working with Virgo and Hafner, Mossbauer resonant absorption studies of ^{57}Fe iron-rich specimens from 10044,25 were carried out at liquid helium temperatures (34). This revealed magnetic hyperfine splitting, and a relatively sharp transition temperature was found to exist between 20°K and 30°K, interpreted as a Neel point. The spin orientations below this point are assumed to be ferrimagnetic. However, the iron-bearing chain silicates are generally not magnetically ordered, even at very low temperatures, especially if the amount of diamagnetic cations (magnesium, calcium) substituting for iron at the octahedrally coordinated positions is larger than 25% as in the iron-rich lunar augite.

This data was then correlated with our high resolution electron microscopy results, and we were able to speculate that the rather unusual magnetic ordering in the lunar pyroxene could be due to iron-iron clustering in the single crystal domain bands. This would imply a substantial enrichment of the bands in iron as compared to the interband regions. (Later magnetic decoration studies of lunar pyroxenes from Apollo 12 and 14 tended to confirm this hypothesis, as will be described subsequently.)

In the domains between the bands, clustering of the Mg,Ca seems likely. If one assumes the extreme case that the ferrous ions are entirely located within the bands, one is led to the pigeonite composition $En_{0.19}Fs_{0.68}Wo_{0.47}$ for the bands and an almost pure diopside of the composition $En_{0.53}Wo_{0.47}$ for the interband domains (molecular percent). If one assumes complete ordering of the Mg and Ca in the bands, the Fe^{2+} site occupancy in M1,M2 is 0.62, 0.73 respectively. This would certainly produce magnetic ordering at low temperatures due to M1-oxygen-M2 superexchanges (34).

b. <u>Apollo 12 Studies.</u> Based on this preliminary work, interdisciplinary investigations were continued on Apollo 12 clinopyroxenes. Cores of yellow-green, calcium-poor pigeonite $Wo_9En_{60}Fs_{32}$ from coarse-grained Apollo 12 lunar basalt 12021 were separated and supplied to us by Virgo, Hafner and Warburton (35). The crystals were again cleaved and sectioned by essentially non-destructive diamond knife ultramicrotomy techniques or fragmented and mounted directly on thin film substrates.

High resolution electron microscopy and diffraction studies were conducted at 290°K, 4.2°K and 1.8°K at voltages ranging from 75 kV to 200 kV. Using a special specimen stage at liquid helium temperatures, reduced radiation damage and contamination were observed. Specimen cooling was provided by the world's only functioning Collins closed-cycle superfluid helium refrigeration system.

Arrays of uniform, electron-dense lamellae of exsolved augite ca. 200Å wide were observed in most of the crystal fragments examined. The bands appeared to be oriented mainly along (001) and (100) and other planes, and they displayed tapered ends, dense borders and relatively light central regions. The bands are separated by wide, less-dense interband regions (ca. 500Å to 600Å) of apparently homogeneous host (36). These single crystal domain bands appeared to be less regular and slightly smaller than the ca. 300Å bands initially observed in the Apollo 11 specimens. Many of them also displayed splitting and serrated or scalloped edges (37).

Contrast changes dependent on the orientation of the specimen to the incident electron beam were evident. This can be ascribed to antiphase domain boundaries in the pigeonite. Bands in certain other regions showed no modifications when the specimen was tilted ca. 1° to 5°, perhaps indicating that they represent localized areas of different compositions.

1) <u>Heating Experiments.</u> Experimental modifications involving heating of Apollo 11 and Apollo 12 specimens were also carried out. Electron optical studies of heated Apollo 11 specimens revealed a splitting of the bands into finer structures, leading finally to obliteration of the exsolution lamellae (36).

Apollo 12 specimens heated by Virgo and his colleagues for 8 days at 1125°C in a 10^{-5} mm Hg vacuum indicated the absence of exsolved augite. However, sharp diffraction patterns could still be observed, indicating a crystalline homogenization. This may be due to a more disordered Mg and Fe distribtuion between the crystal sites (37).

2) <u>Experiments Involving Magnetic Decoration.</u> Through the application of new techniques for the study of ferromagnetic domain boundaries, we have now been able to directly visualize differences between the bands and the interband regions. By condensation of evaporated Fe, small ferromagnetic single crystals can be formed. These preferentially deposit on the traces of Block walls on the surface of ferromagnetic crystals. This technique yields a permanent picture of the domain pattern. In view of the increased penetration power of the 200 kV electron microscope, no replica patterns were needed.

Our preliminary examinations of the "decorated" specimens at room temperature indicated the presence of magnetic domains related to the band structures, probably indicative of an enhanced concentration of iron within the electron dense thin exsolution lamellae. This supports our earlier suggestion that the band structures are similar to images of magnetic domain walls seen in thin layers of ferromagnetic materials (30). It also provides evidence for the assumption that the rather unusual magnetic ordering in the lunar pyroxene could be due to iron-iron clustering in the single crystal domains (34, 38).

The domains and any lattice imperfections can also be directly resolved through the application of cryo-electron microscopy at liquid helium temperatures. In this way, we were able to observe trapped flux patterns by Trauble and Essmann's decoration techniques (39,40) or through the distribution and detection of regular arrays of flux lines in the electron diffraction patterns of the lunar clinopyroxenes.

3) Low Temperature Experiments. Additional studies were carried out at low temperatures in the liquid helium range. Examination of the native yellow-green pigeonite specimens at 1.8^o to 4.2^{o}K revealed substructures in certain local areas which were not present in the corresponding regions of the same specimens at 290^{o}K (36,37). Together with related Lorentz microscopy findings, these results are tentatively interpreted as being consistent with the assumption of greater ordering of iron atoms in these areas.

4) Lunar Cooling History. This observation of extensive exsolution in the apparently homogeneous, calcium-poor pigeonite and correlated Mossbauer absorption studies suggest that this coarse-grained lunar basalt must have been subjected to an exceedingly slow cooling rate in the subsolidous temperature range (41).

Virgo, Hafner and Warburton have observed considerable long range ordering within the crystal fragments at temperatures of 810^o to 480^{o}C. Taken together with the submicroscopic unmixing and ordering of Mg and Fe among the cation sites, this indicates that annealing of the rock from the surface of the moon took place at about 600^{o}C (41). Hawaiian lavaflows and certain other terrestrial orthopyroxenes do not demonstrate this type of characteristic long range ordering when cooled to 480^{o}C. However, similar effects are known in ancient terrestrial igneous rock, particularly in dykes cutting genisses in continental shield regions, where the rock has been rapidly cooled and then reheated or held for long periods of time at moderate temperatures.

b. Studies of Apollo 14 and 15 Samples. Moon rocks from Apollo 15, chemically zoned clinopyroxene 15535 of heterogeneous composition $En_{49.80 \pm 9.90}Fs_{34.46 \pm 5.52}Wo_{15.60 \pm 6.50}$, and pigeonite fraction 15076 of homogeneous composition $En_{65.84 \pm 1.49}Fs_{27.83 \pm 0.90}Wo_{5.83 \pm 0.58}$ were examined by electron optical techniques (42). Our sample 15535 of 16 mg of small grains of chemically zoned clinopyroxene was found to contain different mineral phases, still intergrown. We crushed the grains to a finer size and removed the black opaque particles. The remaining grains were then cleaved further to sizes of 1μ to 5μ in diameter by the cleavage preparation technique. Again, the black impurities were removed with very fine tungsten needles by micro-manipulation, leaving a purer clinopyroxene. The crystalline layers, varying in thickness from 200Å to 600Å, were placed on ultrathin carbon coated formvar films about 150Å thick.

These specimens were examined by both 75 kV and 200 kV electron microscopy and selected-area electron diffraction at room temperature and at 4.2°K. Of all the crystals examined, we observed exsolution lamellae in 20% of sample 15535 and in 32% of sample 15076. The remainder showed no band structures. The lamellar structure consisted of a dense band and an interband spacing which are single crystal domains oriented mainly along the (001) or (100) with uniform boundaries.

In sample 15535, dense bands with widths of 25Å to 550Å (average width 250Å) were observed directly. Corresponding interband regions ranged in width from 50Å to 900Å, with an average width of 330Å. Approximately 43% of the total crystal volume of this specimen sample is dense band.

In pigeonite sample 15076, the width of the dense bands varied from 100Å to 1800Å, with an average width of 1000Å. The interband width ranged from 300Å to 6200Å with an average width of 3100Å. In this specimen sample, approximately 34% of the total crystal volume is dense band.

These measurements of the dense band widths of sample 15535 are slightly smaller than those from Apollo 11 pyroxene 10044,25 which averaged between 250Å and 300Å in width. The interband measurements from sample 15535 were about the same as those from Apollo 11 pyroxene 10044,25, which ranged from ca. 100Å to 700Å, with an average of ca. 330Å. Of the total crystal volume, approximately 43% is dense band in sample 15535 and 49% is dense band in sample 10044,25.

The band widths of specimens from sample 15076, averaging ca. 1000Å in dense band and ca. 3100Å in interband width, are much greater than those measured by the same techniques in Apollo 12 sample 12021. These latter averaged ca. 230Å and ca. 650Å in the dense band and interband respectively. However, the two pigeonite samples show a similarity in their percentage of dense band content of the total crystal volume being 24% in 15076 and 26% in 12021.

A lattice spacing of 2.51Å was also detected within these bands in both of the Apollo 15 specimens examined by selected-area electron diffraction. The spacing is oriented mainly parallel to the (001) or (100). This is also the direction of the domain boundary of the band.

Several researchers have reported extremely thin exsolution lamellae in 15076 pigeonite not more than 1 or 2 unit cells thick (43,44). This is in contrast to our recorded observation of 1000Å wide bands within the specimen sample. Thus our findings may represent a repeat distance of these fine lamellae or some feature other than lamellae which has heretofore been unobserved. It should also be considered that our high resolution electron micrographs possibly depict a small percentage of large exsolution lamellae which are not detectable by the photography techniques used by other researchers.

2. Organometallic Superconducting Compounds. Representative samples of new classes of layered, transition metal dichalcogenide intercalation complexes with unique superconducting properties have been examined in correlated high resolution electron microscopy and diffraction studies. These exceptionally anisotropic, crystalline, metallic compounds, formed by inserting organic molecules of pyridine, aniline, and N,N-Dimethlyaniline between metallic layers of tantalum disulfide, niobium disulfide and niobium diselenide by F. R. Gamble and his colleagues, are bulk superconductors which may have useful high magnetic field properties.

The demonstration of atomically thin metallic layers separated by an organic barrier in our preliminary studies indicated the possibility that this work would have a bearing on the general problem of superconductivity and superconducting tunneling. The multilayered structures of atomic dimensions depicted in the high resolution electron micrographs were well within the range of tunneling phenomena. Following critical evaluation of the results of these studies, we were able to hypothesize that the multi-layered structures might exhibit characteristics related to Josephson tunneling in novel ways uniquely derived from the three-dimensional configuration of these superconducting intercalation complexes (45). Subsequently Dr. Gamble and his colleagues were awarded a grant to further investigate Josephson tunneling in relation to these organometallic compounds.

Extending this early work at room temperatures, improved low temperature instrumentation and preparation techniques have been used to resolve significant details of the atomic lattice in the microcrystalline specimens directly for the first time at 1.8° to 4.2°K and to record the results in color high resolution electron micrographs (46). At these temperatures, characteristic changes have been observed repeatedly in the fine structure of the complexes, and the relationship of these results to the singularly high anisotropic nature of these superconductors is now being investigated.

Recent work has centered on 2H-NbS_2 and related compounds examined by high resolution electron microscopy and diffraction with specimen cooling at 4.2° to 1.8°K. When the thin layers (ca. 200Å to 600Å) of both 2H-NbS_2 and 2H-$NbSe_2$ were imaged with the incident 200 kV electron beam normal to the layers, we have regularly observed characteristic doughnut-shaped structures ca. 100Å to 200Å in diameter. These doughnut-shaped structures have a typical fine structure exhibiting an electron-dense ring of ca. 20Å to 40Å and a light core. In addition, these characteristic ring structures appear only when the specimen is observed at liquid helium temperatures; they disappear as soon as the specimen warms up above 4.2°K and reappear again when the same sample is cooled to 4.2°K (46). (Thus, without the aid of the high resolution cryo-electron microscope system which is now available to us, these structures would never have been visualized.)

In many areas, the doughnut-shaped structures seem to be arranged in regular patterns, but this is only a preliminary observation. However, in view of the reproducibility of these images and results, recorded with specimen cooling at 4.2°K and in a magnetic field of ca. 6000 to 8000 gauss, we believe we may be dealing with electron microscope images of fluxoids in the form of quantized vortexes (33,46,47).

This work was reported in a series of comprehensive papers, including "Electron Microscopy and Diffraction of Layered Superconducting Intercalation Complexes," SCIENCE, Vol. 174, pp. 498-500, plus the front cover illustration of this issue which was one of our high resolution electron micrographs of the superconducting compounds; "Electron Microscopy and Diffraction of Layered Superconducting Intercalation Complexes at Liquid Helium Temperatures," a paper presented at the Office of Naval Research Conference on the Physics and Chemistry of Layered Compounds, Monterey, Calif., Aug., 1972; "Applications of Cryogenics in Electron Microscopy," a paper presented at the annual meeting of the Cryogenics Society of America and the American High Vacuum Society, Chicago, Oct., 1972; and "High Resolution Electron Microscope Studies at Liquid Helium Temperatures," a paper presented at the joint meeting of the Louisiana and Texas Societies for Electron Microscopy, New Orleans, Feb., 1973.

Model experiments are planned with evaporated thin films of lead-bismuth as suggested by Drs. T. Geballe and C. M. Varma (48,49). The results of this research may permit us to further characterize the highly anisotropic nature of these new superconductors.

3. Ultraminiaturized Electronic Circuitry. Using the modified electron microscope with superconducting lenses, we have printed miniaturized electronic circuits on high resolution films and reduced them to the point where over one million working elements can be contained in a single cubic inch. In the human brain, the packing density of the cells is somewhere around 10 to 100 billion elements per cubic inch.

In our laboratory, we have already been able to practically use electron optical techniques to develop printed circuits of the micron and submicron size. Use of similar ultraminiaturized circuits for transmitting functional data recorded within the cellular and subcellular domain of living systems is within the scope of current technology. We can conceive of integrated circuits that begin to approach the dimensions of macromolecular assemblies and can be incorporated into key junctional sites of living nerve membranes or be placed on red blood cells without disturbing the cells. Such submicroscopic prosthetic sensors--with biosynthetically produced protein coats--could form integral parts of the nervous system and serve unique functions. For example, they could harness the natural electricity of the body to produce a direct operational link between the nervous system and man-made information processing systems, such as computers, of similar complexity to serve unique diagnostic functions and perhaps even someday help doctors to restore paralytic nerve damage at the macromolecular level.

By using ultraminiaturized circuitry and new organometallic emulsions to provide superconducting memories, we can also begin to think of achieving switching times on the order of pico-seconds (i.e. 10^{-12}) and designing computers with working elements on the order of megabits to approximate the human brain's complexity and redundancy. By this method of multiplexing, we can also more closely duplicate nature's reliability.

Extensive experimentation has been conducted with miniaturized electronic circuitry at liquid helium temperatures. The results indicate that this approach extends the range of basic limits of microelectronics at room temperatures, particularly a) Resistive direct current attenuation when dealing with extremely small circuit elements, and b) The thermal activation failures and most importantly the electromigration defects which have now been found to set in especially in metal lines less than 1 micron wide where typical current densities of ca. 10 million amperes per square centimeter lead to breakdown of the circuits.

Results of our recent studies, carried out in part under NASA NGL 14-001-012 and presented at the 18th Annual International Electron Devices Meeting in Washington (50), also indicate that this approach may lead to the observation of new physical and physical-chemical phenomena which could in turn open the way to new fabrication technologies and related systems applications for microelectronic circuitry (33).

4. Information Storage and Retrieval. Using the modified electron microscope and various light optical approaches, together with the organometallic emulsions and fly's eye lenses which we have developed and perfected in the course of this project, we have been able to make vast strides in solving some of the problems of information storage and retrieval.

In an area of the size of an ordinary microscope slide, an array of the tiny lenses can contain the equivalent of several complete books stored page by page (each reduced by factors of from 1:1000 to 1:50,000 in a single electron optical operation) in the focal point of each fly's eye lens. By combining this with the new types of recording media, we have been able to reduce both color and black and white printed images to a point where 500 to 1600 full size (i.e. 8 1/2 x 11") pages can be stored in the area of one square inch. This represents a significant miniaturization of the conventional microfiche, and such reductions have already been experimentally achieved on a small scale in our laboratory.

Such a storage system has the advantage of being a virtually permanent process since the photographically reduced or electron optically demagnified images can be placed in the focal plane of the individual lenses in a permanent embedding process. Because the stored data would be virtually invulnerable to scratching and other types of "normal" deterioration which affects microfilm during ordinary readout, this system may well be much more practical.

Functioning prototypes for both pico-cameras and pico-projectors have been developed, incorporating the preliminary developments in new recording media, fly's eye lenses and light and electron optics. In addition, the lenses have been adapted to an experimental, semi-automatic contact printing device. With this system, it has been possible to immediately make up to 10 copies of a microfiche while simultaneously reducing it by factors of roughly 1:10 and recording it on the various new media.

The further perfection of these types of hand-held, battery operated devices and of their component parts would not only markedly reduce the amount of space required for storing data, but it would also be an indispensible requirement for converting the present microfiche stores to more efficient ones based on high resolution light and electron optics. It might also pave the way for the development of automatic information storage and retrieval systems.

Preliminary experiments have demonstrated that the new types of recording media which we are using record amplitude and phase as well as frequency, making them ideal for color holographic recording on a level which has never before been attained. Based on this work and on my own earlier investigations into the structure and mechanisms of the human brain, we can now begin to think in terms of developing new readout systems for ultraminiaturized black and white and color data based on holography which could begin to rival the elegance, speed and efficiency of the most remarkable information processer--the memory portion of the human brain.

5. Studies of the Submicroscopic Structure of Cell Membranes and Related Biological Systems.

a. Hydrated Membrane Structure. Correlated ultrastructural and biochemical studies over the past few years have revealed certain general characteristics of membrane organization. Coherent paucimolecular layers of indefinite lateral extension appear to consist of a periodic, hydrated lipoprotein substrate which is integrated with specific macromolecular repeating subunits organized in asymmetric paracrystalline arrays within the plane of the layers (3,4).

Our work with David Green and his colleagues demonstrated that membranes are made up by the stereospecific association of macromolecular repeating units of lipoprotein whose conformations are dependent on their association with the membrane substrate (51). Many lipoprotein membranes, both native and artificial, respond in vivo and in vitro to the binding of specific ligands by some modification of their properties. This process reflects the rearrangement of the membrane organization and, presumably, of the repeating unit conformation.

Furthering this work, we have carried out collaborative studies of humming bird heart mitochondria with Dr. L. J. DiDio of the Medical College of Ohio at Toledo. We have also undertaken a series of preliminary studies of cytochrome oxidase in collaboration with Drs. David Green and Rod Capaldi of the University of Wisconsin. The resulting high resolution electron micrographs and electron diffraction patterns of these latter highly ordered specimens have now given us a clearer picture of these enzymic complexes than we had previously. We are now in the process of trying to devise an extensive series of experiments designed to study the crystalline state of the oxidase under hydrous conditions.

Systematically applying high voltage cryo-electron microscopy, diamond knife cryo-ultramicrotomy and related electron optical developments, we have been able to approach the native hydrated state in investigations of various biological specimens, directly observing increasing amounts of meaningful information and structural detail (3,16,52).

Specimens of DNA, nerve myelin, cock retinal rod outer segments and bacteriophages have been sandwiched between impermeable ultra-thin films in specially-adapted vacuum-tight microchambers to trap their hydrated components at liquid helium temperatures. Data indicate that new details of the hydrated membrane structure and dimensions can be visualized with resolutions of ca. 8Å to 16Å. While these results are only preliminary in nature and are still being evaluated, they do indicate the potential of the cryo-electron microscope in biological and biomedical studies of hydrated systems (16,52).

b. Ice Crystal Formation and Ordered Water. Based on earlier work (53), and on recent technical improvements to overcome the limitations of vacuum sublimination, electron bombardment and contamination, it has been possible to directly study ice-crystal formation and growth. With special shielding devices to protect the specimens from contamination, we have been able to record electron micrographs and electron diffraction patterns of minute ice crystals which have been stabilized by cooling.

Critical investigations have recently been carried out on ice crystals formed by the deposition of water vapor on cold, thin carbon and single crystal gold films in order to determine the existence of cubic and other ice modifications at temperatures of 1.8° to $4.2^{\circ}K$. The results of these studies will have a great implication for the preservation of biological systems at low temperatures and the state of water in frozen materials. In addition, they may also have a direct bearing on the concept of selective permeability of nerve membranes envisaged in terms of molecular "pores" lined with highly ordered water.

High resolution color electron micrographs recording the results of this work have been published in the Annals of the New York Academy of Sciences (3) and used as the front cover illustration of a recent issue of the Journal of the American Medical Association.

c. Cell Membrane Ultrastructure, Viruses and Cancer. Since the most naturally-occurring animal cancers to which viruses have been linked involve an RNA virus rather than a DNA one, the structural characterization of viral enzymes takes on added importance. With this in mind, we have begun to examine the reverse transcriptase enzymes of MSV-MLV complexes provided by Dr. Joseph L. Melnick and his colleagues at Baylor University Medical School. In the course of this work, we have employed the techniques and experience which we gained in our earlier extensive studies of RNA polymerase molecules from E. coli (54).

Pursuing collateral research, and presuming that the reverse transcriptase is not a single enzyme, but rather a multienzyme complex as some researchers have hypothesized, we are planning to apply the sophisticated correlated techniques developed in the course of our studies of the pyruvate dehydrogenase complex (including micro-droplet cross-spraying, high resolution electron microscopy and biochemical analysis) (55).

In preliminary studies of purified RNA-dependent DNA polymerase of the MSV-MLV complex, a definite ring structure of ca. 60Å to 80Å in diameter characteristic of DNA polymerase was revealed.

In addition, fresh, purified preparations of MSV-MLV which were never frozen were used in high voltage ultrastructural studies of whole mount preparations. A spherical nucleoid and inner core of the virion were more clearly resolved in the color images than in the black and white high resolution electron micrographs, and two tracks in the core were resolved. In addition, further details of the internal components were clearly visible in the whole mount preparations.

Both rod and tadpole shaped oncornavirus particles were observed under identical conditions and recorded in both black and white and color high resolution electron micrographs. The tadpole shaped particles were found in fresh preparations of the unfrozen virus, fixed either in 1-2% phosphotungstic acid, pH 7.2, or in buffered, pH 2.2, 1-3% glutaraldehyde. The tadpole shaped virus heads seem to be angular and may be structurally similar to those previously published by other investigators, and they contain small globular and cuboid subunits.

This preliminary work was presented at the Second Duran-Reynals International Symposium on Viral Replication and Cancer in Barcelona (16) and is the subject of a paper "Fine Structure of the Murine Sarcoma-Leukemia Virus as Revealed by High Resolution Electron Microscopy and Color Interferometry" which is to be published in the Journal of the National Cancer Institute (56).

REFERENCES

1. Fernandez-Moran, H. (1953): Exptl. Cell Res., 5, 255-56.
2. Fernandez-Moran, H. (1960): J. Appl. Phys., 31, 1840.
3. Fernandez-Moran, H. (1972): Annals of N.Y. Acad. Sci., 195, 376-89.
4. Fernandez-Moran, H. (1973): "Applications of Cryogenics in Electron Microscopy" in Applications of Cryogenic Technology, Vol. 5. Whitestone: Scholium International, Inc., pp. 153-81.
5. Fernandez-Moran, H. (1966): Proc. 6th Internatl. Cong. E.M., Tokyo, Japan, 1, 13,47,147.
6. Fernandez-Moran, H. (1965): Proc. Natl. Acad. Sci., U.S., 53, 445-51.
7. Fernandez-Moran, H. (1966): Proc. Natl. Acad. Sci., U.S., 56, 801-08.
8. Fernandez-Moran, H. (1972): "Die molekulare Struktur von Zellmembranen" and "Elektronenmikroskopie von Supraleitern bei Temperaturen von flussigen Helium." papers presented at the

Institut fur Theoretische Physik der Technischen Hochschule Darmstadt, Darmstadt, West Germany, June, 1972.

9. Fernandez-Moran, H. (1972): "Molekulare Struktur von Zellmembranen" and "Electronenmikroskopie von Supraleitern bie Temperaturen von flussigen Helium." papers presented at Biophysics Colloquium, Institut for Biochemie, Gesellschaft fur Strahlen und umweltforschung MbH, West Germany, June, 1972.

10. Fernandez-Moran, H. (1970): Proc. 7th Internatl. Cong. E.M., Grenoble, France, 2, 91-92.

11. Fernandez-Moran, H., Ohtsuki, M., and Hough, C. (1970): Proc. 7th Internatl. Cong. E.M., Grenoble, France, 3, 9-10.

12. Dodd, M.G. (1972): Proc. 5th European Cong. on E.M., pp. 564-65. Cambridge, England.

13. Septier, A. (1972): Proc. 5th European Cong. on E.M., pp. 104-09. Cambridge, England.

14. Uyeda, N., Kobayashi, T., Ohara, M., Watanade, M., Taoka, T., and Harada, Y. (1972): Proc. 5th European Cong. on E.M., pp. 566-67. Cambridge, England.

15. Fernandez-Moran, H. (1972): "High Resolution Electron Microscopy of Motor Nerve Cells" paper presented at the Annual Convention of the Kiwanis, Board of Trustees of the Spastic Paralysis Research Foundation, Peoria, Ill., Aug. 19, 1972.

16. Fernandez-Moran, H. (1973): "High Voltage, Low-Temperature Electron Microscope Studies of Virus Structure." in Viral Replication and Cancer: Proc. 2nd Duran-Reynals International Symposium. J. L. Melnick, S. Ochoa and J. Oro, eds. Barcelona: Editorial Labor, S.A., pp. 69-94.

17. Fernandez-Moran, H. (1966): The Neurosciences: A Study Program. G. Quarton, T. Melnechuk, F. O. Schmitt, eds. New York: Rockefeller University Press, 281ff.

18. Dubochet, J., Duchommun, M., Zollinger, M., and Kellenberger, E. (1971): J. Ultrastruct. Res., 25, 147ff.

19. Farley, M. (1972): Microstructures, 3, #3, 13.

20. Moses, R. W. Jr. (1973): "Procedures for Correcting Aberrations in the High Voltage Electron Microscope." Proceedings of the Royal Society, London. In Press.

21. Daunt, J. G. (1970): Proc. Workshop on Naval Applications of Superconductivity, NRL Report 7302 Washington: Naval Research Laboratory. 40-61.

22. Fernandez-Moran, H. (1970): Exptl. Cell Res., 62, 90-101.

23. Fernandez-Moran, H. and Rowe, S. J. (1972): Hearings on Energy Research and Development, Subcommittee on Science, Research and Development, Committee on Science and Astronautics, U.S. House of Representatives, 92nd. Cong., 2nd. sess., #24. Washington: U.S. Govt. Printing Office . pp. 461-68.

24. Mollenstedt, G., and Speidel, R. (1960): Phys. Blat., 16, 192-93.

25. Fernandez-Moran, H. (1966): Biology and the Exploration of Mars. Washington: National Research Council of the National Science Foundation, Publ. #1296, pp. 503-06.

26.

27. Fernandez-Moran, H. (1974): "Seeing the World of the Invisible: Now and in the Future." Chicago: Encylcopedia Britannica. To be published in YEARBOOK OF SCIENCE AND THE FUTURE, now in press.

28. Kanaya, K., Kawaktsu, H., Atoda, N., Yotsumoto, H., and Ono, A. (1970): Proc. 7th Internatl. Cong. E.M., Grenoble, France, 1, 359-60.

29. Nathan, R. (1969): Personal Communication.

30. Fernandez-Moran, H., Ohtsuki, M., Hafner, S. S. and Virgo, D. (1970): Proc. Apollo 11 Lunar Sci. Conf., Geochim. Cosmochim. Acta Suppl. 1, Vol. 1, pp. 409-17.

31. Radcliff, S. W., Heuer, A.H., Fisher, R.M., Lally, J.S., Christie, J.M., and Griggs, D.T. (1970): Proc. 7th Internatl. Cong. on E.M., Grenoble, France, 2, 637-38.

32. Bailey, J.C., Champness, A.C., Dunham, A.C., Esson, J., Fyfe, W.S., Mackenzie, W.S., Stumpfl, E.F., and Zussman, J. (1970): Science, 167, 592-94.

33. Ross, M., Bence, A.E., Dwornik, E.J., Clark, J.R., and Papike, J. J. (1970): Science, 167, 628-30.

34. Fernandez-Moran, H., Hafner, S.S., Ohtsuki, M., and Virgo, D. (1970): Science, 167, 686-88.

35. Hafner, S.S., Virgo, D., and Warburton,D. (1971): Proc. 2nd Lunar Sci. Conf., Geochim. Cosmochim. Acta Suppl. 2, Vol. 1, pp. 91-108.

36. Fernandez-Moran, H., Ohtsuki, M., and Hibino, A. (1971): Proc. 2nd Lunar Sci. Conf., Geochim. Cosmochim. Acta Suppl. 2, Vol. 1, pp. 109-16.

37. Fernandez-Moran, H., Ohtsuki, M., and Hough, C. (1972): "Correlated Electron Microscopy and Diffraction of Clinopyroxenes from Apollo Rocks." paper presented at the 3rd Lunar Science Conference, Houston, Tex., Jan., 1972.

38. Virgo, D. (1973): Personal Communication.

39. Trauble, H., and Essman, U. (1966): J. Sci. Instru., 43, 344.

40. Trauble, H., and Essman, U. (1968): J. Appl. Phys., 39, 4052-59.

41. Hafner, S.S., Virgo, D., Warburton, D., Fernandez-Moran, H., Ohtsuki, M., and Hibino, A. (1971): Nature, 231, #21.

42. Fernandez-Moran, H., Virgo, D., and Ohtsuki, M. (1973): 4th Lunar Science Conference Abstracts, pp. 236-38.

43. Jagodzinski, H., and Korekawa, M. (1973): 4th Lunar Science Conference Abstracts, pp. 409-11.

44. Kline, D., Kolopus, J.L., and Weeks, R.A. (1972): "Nuclear Magnetic Resonsnce Properties of Returned Lunar Samples." paper presented at the 3rd. Lunar Science Conference, Houston, Tex., Jan., 1972.

45. Fernandez-Moran, H., Ohtsuki, M., Hibino, A., and Hough, C. (1971): Science, 174, 498-500.

46. Fernandez-Moran, H. (1972): "Electron Microscopy and Diffraction of Layered Superconducting Intercalation Complexes at Liquid Helium Temperatures" paper presented at Office of Naval Research Conference on the Physics and Chemistry of Layered Compounds, Monterey, Calif., Aug. 17-19, 1972.

47. Fernandez-Moran, H. (1973): "High Resolution Electron Microscopy Studies at Liquid Helium Temperatures" paper presented at the Joint Meeting of the Louisiana and Texas Societies for Electron Microscopy, New Orleans, La., Feb. 8, 1973.

48. Geballe, T. (1972): Personal Communication.

49. Varma, C.M. (1972): Personal Communication.

50. Fernandez-Moran, H. (1972): "Microelectronics at Liquid Helium Temperatures" paper presented at 18th Annual International Electron Devices Conference, Washington, D.C., Dec. 4, 1972.

51. Fernandez-Moran, H., Oda, T., Blair, P.V., and Green, D.E. (1964): J. Cell Biol., 22, 63-100.

52. Fernandez-Moran, H. (1973): "High Resolution Electron Microscopy Studies at Liquid Helium Temperatures" paper presented at the Joint Meeting of the Louisiana and Texas Societies for Electron Microscopy, New Orleans, La., Feb. 8, 1973.

53. Fernandez-Moran, H. (1965): Annals of N.Y. Acad. Sci., 125, 739-52.

54. Colvill, A.J.E., van Bruggen, E.F.J., and Fernandez-Moran, H. (1966): J. Mol. Biol., 16, 191ff.

55. Fernandez-Moran, H., Reed, L.J., Koike, M., and Willms, C.R. (1964): Science, 145, 773.

56. Fernandez-Moran, H., Melnick, J.L., Biswal, N., and Hough, C. (1974): "Fine Structure of the Murine Sarcoma-Leukemia Virus as Revealed by High Resolution Electron Microscopy and Color Interferometry" J. Natl. Cancer Instit., In Press.

LECTURES AND PUBLICATIONS DURING FUNDING PERIOD

1. Fernandez-Moran, H., Melnick, J. L., Biswal, N., and Hough, C. (1974): Fine Structure of the Murine Sarcoma-Leukemia Virus as Revealed by High Resolution Electron Microscopy and Color Interferometry, J. Natl. Cancer Inst., In Press.

2. Fernandez-Moran, H. (1974): Seeing the World of the Invisible: Now and in the Future in Britannica Yearbook of Science and the Future 1975. Chicago: Encylcopedia Britannica. In Press.

3. Fernandez-Moran, H. (1973): Applications of Cryogenics in Electron Microscopy in Applications of Cryogenic Technology, V. Robert Carr, ed. Whitestone: Scholium International, Inc. pp. 153-81.

4. Fernandez-Moran, H. (1973): High Voltage Low Temperature Electron Microscope Studies of Virus Structure in Viral Replication and Cancer: Proc. 2nd Duran-Reynals Internatl. Symposium. J. L. Melnick, S. Ochoa and J. Oro, eds. Barcelona: Editorial Labor, S.A. pp. 69-94.

5. Fernandez-Moran, H. "Chemistry--A Glimpse Into the Future." Paper presented at the Golden Anniversary Meeting of the American Institute of Chemists, Houston, May 17-19, 1973.

6. Fernandez-Moran, H., Ohtsuki, M., and Hough, C. "High Resolution Electron Microscopy Studies at Liquid Helium Temperatures." Paper presented at the Joint Meeting of the Louisianna and Texas Societies for Electron Microscopy, New Orleans, Feb. 8, 1973.

7. Fernandez-Moran, H., Ohtsuki, M., and Hough, C. "Correlated Electron Microscopy and Diffraction of Clinopyroxenes from Apollo Rocks." Paper presented at the 4th Lunar Science Conference, Houston, Jan. 11-14, 1973.

8. Fernandez-Moran, H. (1972): "Microelectronics at Liquid Helium Temperatures." Paper presented at 18th International Electron Devices Conference, Washington, D.C., Dec. 4, 1972.

9. Fernandez-Moran, H. (1972): Advances in Electron Microscopy." Paper presented at Texas Society for Electron Microscopy Fall Symposium, San Antonio, Oct. 28, 1972.

10. Fernandez-Moran, H. (1973): "Cell Ultrastructure." Two week intensive study course presented in Visiting Distinguished Professor Program, Texas A & M University, College Station. Oct. 15-27, 1972.

11. Fernandez-Moran, H. (1972): "Leroy Augenstein Memorial Lecture in Biophysics." Presented at the occasion of the dedication of the Augenstein Memorial Library, Michigan State University, East Lansing, Sept. 25, 1972.

12. Richard S. Lewis. "Humberto Fernandez-Moran." in Science Year 1973. Chicago: Field Enterprises Educational Corp. pp. 382-97.

APPOINTMENTS

1. Member of the Board, Pan American Medical Association, Inc., 1973.

2. Member, Board of Scientific Advisors, Yerkes Primate Research Center, Emory University, Atlanta, Georgia, 1972.

3. Honorary Member, Pan American Association of Anatomists, 1972.

COLLABORATION WITH OTHER RESEARCHERS DURING FUNDING PERIOD

1. Dr. David Virgo, Carnegie Institution Geophysical Laboratories, Washington, D.C.
   High resolution electron microscopy and diffraction studies of lunar pyroxenes.
2. Dr. J. L. Melnick, Dr. N. Biswal and associates, Baylor University Medical Center, Houston.
   High resolution electron microscopy of 60-70S RNA and reverse transcriptase from MSV-MLV complexes.
3. Dr. David Green, The University of Wisconsin.
   High voltage low temperature electron microscopy and diffraction studies of cytochrome oxidase specimens from mitochondria.
4. Dr. Liberato J. A. DiDio, The Medical College of Ohio at Toledo.
   High voltage electron microscopy studies of freshly prepared hummingbird heart mitochondria at room and liquid helium temperatures.
5. Mr. J. D. Ratcliff, Reader's Digest.
   Collaboration on article "I Am Joe's Cell" (December, 1973, p. 120).